

1.0 SCIENTIFIC ABSTRACT OF THE PROTOCOL

Cystic fibrosis (CF), the most common lethal genetic disease in North America, is caused by defects in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The CFTR gene product is required for regulation of epithelial chloride transport in multiple organs, including the airways. Cystic fibrosis lung disease develops gradually over many years as abnormally viscous secretions that lead to airway obstruction, infection, inflammation, and fibrosis. It ultimately may lead to respiratory failure, which is the cause of death in greater than 90% of CF patients. It is thought that correction of the underlying CFTR gene defect may result in therapeutic effect on the progressive lung disease.

The vector (tgAAVCF) is derived from Adeno-Associated Virus (AAV), which is not known to cause human disease. This transducing vector can be generated at sufficiently high titers that it is practical as a delivery system and vector preparation can be purified to near homogeneity, removing contaminants and process materials.

Furthermore, AAV-CFTR vectors transduce and express recombinant CFTR *in vivo* after delivery to the airway surface of animals. Long-term vector expression, up to 6 months after a single-dose administration, has been observed in the New Zealand white rabbit and rhesus monkey models. Administration of higher doses of tgAAVCF by aerosol inhalation to rhesus macaques has demonstrated dose-dependent gene transfer and gene expression. Repeat dose administrations at these higher doses were well tolerated, and dose dependent gene transfer was observed. In some animals, serum neutralizing antibody titers to AAV2 increased and low titers of antibody and an elevation of lymphocytes were observed in bronchial wash fluid. There was no other clinical pathology or histologic findings related to the exposure of vector. Biodistribution of the vector to the gonads was not observed.

Over 60 cystic fibrosis patients, to date, have been administered single doses of tgAAVCF with no serious adverse events. Data from administration to the maxillary sinus, nose, lung lobe, and whole lung have shown a dose dependent, persistent gene transfer following single dose administration. A number of functional measurements, including sinus potential difference measurements and changes in

IL-8 levels are suggestive of biological activity, although vector messenger RNA (mRNA) has not yet been detected in human clinical trials to date.

The CF airway has many potential barriers to gene transfer including thick mucous and increased levels of proteases and inflammatory mediators. The primary endpoint of the study is to evaluate the effect of anti-inflammatory and anti-protease pretreatment on airway transduction by aerosolized tgAAVCF. Secondary endpoints include an evaluation of the effect of anti-inflammatory and anti-protease pretreatment and tgAAVCF administration on lung function, and evaluation of any changes in the safety profile of tgAAVCF when combined with pretreatment regimens.

This is a Phase I, randomized, open-label study. All patients will receive the same dose [2×10^{12} DRP] of tgAAVCF vector. The dose of vector is lower than the maximal dose administered in previous trials, allowing for evaluation of changes in transduction, both positive or negative. Patients will be randomized to one of three pretreatment regimen groups:

STUDY GROUP TREATMENTS

Group	Pretreatment	tgAAVCF
1	♦ No pre-treatment	2×10^{12} DRP
2	♦ One aminoglycoside ¹ for 14 days prior to tgAAVCF ♦ One beta-lactam ² for 14 days prior to tgAAVCF ♦ Chest physiotherapy for 14 days prior to tgAAVCF ♦ RhDNase 2.5 mg by nebulizer for 12 days ending 2 days prior to tgAAVCF ♦ Prednisone, 60mg by mouth for 5 days ending day of tgAAVCF	2×10^{12} DRP
3	♦ Prolastin® 250 mg twice a day by aerosol administration ♦ One aminoglycoside ¹ for 14 days prior to tgAAVCF ♦ One beta-lactam ² for 14 days prior to tgAAVCF ♦ Chest physiotherapy for 14 days prior to tgAAVCF ♦ RhDNase 2.5 mg by nebulizer for 12 days ending 2 days prior to tgAAVCF ♦ Prednisone, 60 mg by mouth for 5 days ending day of tgAAVCF	2×10^{12} DRP

¹ Usually Tobramycin. but may be altered based on sputum sensitivities. Tobramycin will be delivered IV 7 mg/kg/day divided every 8 hrs OR based on previous therapeutic monitoring.

² Usually cefepime, but may be altered based on sputum sensitivities Cefepime is delivered 300 mg/kg/day divided every 8 hours.

A total of 15 adult patients are planned to be enrolled. Patients will be randomized to one of three pretreatment regimen groups. Patients will be involved in study procedures for a total

of 10 weeks. Patients will be randomized to a pretreatment regimen and will undergo screening evaluation prior to the induced sputum and baseline bronchoscopy on study day 0. The pretreatment regimen will begin on study day 14, and induced sputum collected on study days 14 and 27. tgAAVCF will be administered on study day 28. Patients will be followed up for safety evaluation and induced sputum collection on study day 42. Bronchoscopy and safety evaluations will be performed on study day 56. Patients with ongoing adverse event(s) at the time of this last visit will be asked to return for further safety follow-up visits until the event has resolved or stabilized.